of a second compound comprising TGF-β is provided in Example 6 on page 49. "causing the compound to access" has been replaced by " contacting the cells with" so as to more specifically describe the invention. Support for new claims 75-81 can be found on page 17, lines 25-27 and page 19, line 11-13. In claim 76, support for the contacting the cells with an embryo's extraembryonic tissue derived compound" may be found on page 34, lines 17-21. In claim 78, support for a method for identifying a compound and for "screening a library of compounds" may be found on page 19, lines12-13. There are numerous assays provided in the application for use in screening libraries, for example on pages 14-19 of the application.

The Examiner has objected to the disclosure because of certain formalities. These formalities have been addressed. With respect to the sequences in the specification, all sequences have been assigned an identifier by amendment. Applicants submitted an electronic version and a paper copy of a listing of 26 sequences on June 30, 1999. Applicants assert that this submission is a complete set of sequences for the above application.

With respect to the drawings; applicants have made the amendments suggested by the Examiner on page 4, line 20- page 5, line2 of the action.

Applicants here submit a substitute declaration with the correct filing date.

With respect to the specification, applicants have made the amendments requested by the Examiner on page 5, lines 5-10 of the action.

With respect to "incorporation by reference", applicants thank the Examiner for pointing out two international applications incorporated by reference. Applicants have amended the specification so as to insert subject matter on hedgehog homologs from WO 95/18856 on page 18, lines 26-33 and page 20, lines 25-30. It is believed that the claims are fully supported by the specification and the knowledge of one ordinarily skilled in the art.

With respect to an Abstract, this has been appended to this response.

The Examiner has rejected claims 1-26 under 35 U.S.C.§112 first paragraph asserting insufficient description and enablement of the invention; and under 35 U.S.C. §112 second paragraph asserting indefiniteness. The Examiner has further rejected claims 1-4, 12, 14-16, 20, 24 and 26 as being anticipated under 35.U.S.C. §102 citing three references- Hemmati-brivanlou et al., Ziegler et al. and Carmaliet et al.

Applicants believe that it would assist the Examiner to summarize the claimed invention.

The applicants' invention is a novel method to stimulate undifferentiated mesodermal derived cells to undergo at least one of hematopoiesis, endothelial cell differential and endothelial cell proliferation. As part of this method, applicants identified for the first time that extraembryonic tissue is a source of molecules suitable for stimulating a population of undifferentiated mammalian mesodermally derived cells to differentiate into cells of the hemato-vascular system including at least one of hematopoiesis, endothelial cell proliferation and endothelial cell differentiation. By analyzing molecules expressed in extraembryonic tissue using novel assays, applicants identified new biological functions for compounds which may be but are not limited to known compositions described in tissue other than extraembryonic tissue. For example, while hedgehog protein is a known protein, it has never before been recognized that hedgehog protein is capable of hematopoiesis or vascular growth (including endothelial differentiation and proliferation).

In early stage embryos, the production of the blood system has two components-blood cell development and the vasculature to transport the blood around the developing embryo. Furthermore, these events are closely tied. The pathway for the development of both processes are described on page 13 , lines 6-15 of the application.

> Blood islands first appear in extraembryonic mesoderm at 7.5dpc [in mice]. .....
> The central cells of the blood islands differentiate into embryonic blood cells. As the blood islands grow, they eventually merge to form a capillary network and the vitelline vessels.."

Applicants have shown that processes of hematopoeisis, endothelial cell proliferation and endothelial cell differentiation are stimulated by compounds in the extraembryonic tissue that cause erythroblasts and endothelial cells to form from undifferentiated precursor cells. (Page 14, 3-24). This has been achieved using a variety of different types of assays "for screening and identifying factors involved in hematopoiesis and vascular growth" (page 15, lines 6-7).

Markers of early blood development (erythroblasts and endothelial cells) were followed in the epiblast culture assay. The markers included ε-globin which is associated with differentiated erythroblasts, GATA-1 which is a transcription factor found in early erythroid progenitors and in differentiated erythroid cells, CD34 which is found in hematopoietic stem

cells, and PECAM-1, flk-1, Vezf-1 (endothelial markers) (page 15, lines 13-15). These data showed differentiation and proliferation of hematopoeitic stem cells and endothelial cells.

Applicants teach that blood island formation arises from hematopoiesis and endothelial cell differentiation and proliferation and is dependent on compounds derived from the extraembryonic tissue. This teaching is supported by add back experiments. For example, epiblasts stripped of visceral endoderm prior to blood island formation failed to stain positively for the markers before or after cultivation and that this activity could be reconstituted by adding back visceral endoderm. Similar findings were obtained using explants or embryoid bodies (page 17, line 7- page 18, line 29). The embryoid bodies contain several cell types including blood cells and endothelial precursor cells. "Knock-out" mutations did not produce blood (or vasculature) while these functions could be recovered by adding compounds isolated from the extraembryonic tissue. Example 2A(a) reveals that lac Z-positive blood islands were easily detected in whole embryo but were absent in epiblast cultures.

Applicants have demonstrated in numerous different ways how at least one of hematopoiesis, endothelial cell proliferation and endothelial cell differentiation arises in response to compounds derived from extraembryonic tissue. With respect to endothelial cell proliferation and differentiation, example 2(B) (ii)(c) describes how visceral endoderm can reprogram the anterior embryonic ectoderm of the epiblast to express both hematopoietic and vascular endothelial markers. (Page 38, line 23-page 39, line 13). Also see Figures 16-3. In Example 2(C), blastosacs are described which when stained revealed "vascular channels" (Fig.3D) (page 42, lines 10-13). Figure 16-3 shows the appearance and then increase in amount of vascular endothelial cell markers that demonstrate that compounds from the extraembryonic tissue stimulate differentiation and proliferation of these cells.

Rejection under 35 USC §102

The Examiner has rejected claims 1-4, 14, 15, 20 and 26 under 35 U.S.C.§ 102(b) as being anticipated by Hemmati-Brivanlou et al. (1995), claims 1-4, 14-16, 20, 24 and 26 under 35 USC 102(b) as being anticipated by Ziegler et al. (1994) and claims 1-2,12 and 20 under 35

U.S.C.§ 102(a) as anticipated by Carmeliet et al (1996). The Examiner has asserted that the 1994 and 1995 references are printed publications in this country that describe the invention and are dated more than one year prior to the date of application for patent in the United States. In addition, the Examiner asserts a more recent third reference dated 1996 as describing the applicants' invention before the applicants made their invention.

It is noted that MPEP 2131.01 states that only one reference should be used in making a rejection under 35 U.S.C.§ 102. None of the three reasons stated in MPEP 2131 for permitting the use of more than one reference in a 102 rejection appears to be relevant here. Moreover, "A claim is anticipated only if each and every element as set forth in the claim is found either expressly or inherently described in a single prior art reference" Verdegaal Bros. V. Union Oil Co. of California, 814 F.2d 628,631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). "The identical invention must be shown in as complete detail as is contained in the ...claim." Richardson v. Suzuki Motor Co.., 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

A claim is anticipated only if each and every element as set forth in the claim is found either expressly or inherently described in a single prior art reference

None of the references cited by the Examiner describe each and every element of the claimed invention .

1) Hemmati-Brivanlou et al. The reference fails to anticipate the claims of the invention because it describes Xenopus development not mammalian development and therefore is not specifically related to hematopoiesis or endothelial proliferation and differentiation in mammals. Moreover, Xenopus does not have extraembryonic tissue and consequently Xenopus stimulation of hematopoiesis or endothelial proliferation and differentiation must occur in a manner that is substantially different from mammals. There is no suggestion in the reference that Xenopus BMP-2 and 4 be used as an optional second compound as described in the claims and taught in Example 6 of the applicants' claimed invention, using mammalian BMP.

(2) The Ziegler reference is directed to thrombopoietin (TPO) which is a megakaryocytes regulatory factor and induces proliferation of murine stem cell populations and production of megakaryocytes and proplatelets in what is described as thrombopoiesis. In the discussion of the

reference, the reference states: "[I]n context of stem cell suspension cultures, the predominant effects of TPO are undoubtedly on megakaryocytopoiesis and thrombopoiesis." The specificity that is sought and indeed described by the authors of the reference with respect to megakaryocytopoiesis and thrombopoiesis, teaches away from the applicant's invention which is directed to a method of stimulating at least one of hematopoiesis, endothelial proliferation and differentiation. The Ziegler reference does not describe or suggest stimulation of at least one of hematopoiesis, endothelial cell proliferation and differentiation.

(3) Carmeliet et al. reference teaches away from providing a compound to stimulate at least one of hematopoiesis, endothelial cell proliferation and differentiation because the reference is not directed to a method of selecting a compound and contacting cells with the compound. Instead, the reference describes morphological changes in heterozygous and homozygous VEGF deficient embryos in which the VEGF gene has been knocked out by targeted inactivation. There is no suggestion in the reference of the effect of adding VEGF or any other compound derived from a particular source to a target cell population to achieve a specific effect.

The Examiner states that the reference causes "VEGF to enter the cells by making embryos which express VEGF". However, it is apparent that all embryos express VEGF unless they are mutated so as to be unable to express VEGF. The authors of the reference have not "caused" a natural process *in vivo* but have only observed the consequences of interfering with this process by disrupting it. This is quite different from the claimed invention which requires contacting a population of cells with an embryo's extraembryonic tissue derived compound.

The Examiner has rejected the claims on the basis of the bridging sentence on page 437-438 which states:

> Immature development and defective interconnections of the embryonic vasculature in $F_1VEGF^{+/-}$ embryos were confirmed by LacZ staining of whole mount $F_1VEGF^{+/-}$ itie-1-lacZ+/- embryos, which revealed a decreased density of sprouting intersomitic and head mesenchyme vessels (Fig 3a,3b) and by injection of ink in the heart which made visible the dorsal aorta and head vessels in VEGF+/- T-ES cell derived embryos but revealed no connection between the heart and the vessel system in VEGF+/- T-ES cell derived embryos (Fig 3c,3d).

This citation does not describe a method of stimulating undifferentiated mammalian mesodermally derived cells. It does not describe any of hematopoiesis endothelial proliferation or differentiation and it does not describe a compound derived from an embryos extraembryonic tissue. The Examiner further cited Table 1 as describing the claimed invention. Table 1 is a summary of the morphological analysis in VEGF deficient heterozygotes and as such is directed away from the claimed invention.

For the above reasons, applicants respectfully submit that the rejections based on novelty should be reversed.

Rejection under 35 U.S.C.112 First Paragraph

Applicants have provided a written description of the method of the invention that would permit one skilled in the art to reproduce and use the method of the invention and has set forth the best mode contemplated by the inventor.

The Examiner has rejected claims 1-6 and claims 9-26 as containing subject matter not described in the specification in such a manner as would show possession of the invention under 35 U.S.C. 112 first paragraph. The elements of the claims are supported by the description of the invention in the specification that includes 6 examples and 17 figures. The specification describes for the first time how compounds from extraembryonic tissue may be used to stimulate at least one of hematopoiesis, endothelial cell proliferation and differentiation in undifferentiated mesodermal cells. This novel finding is significant because the site of expression and timing of expression is important to biological activity. (Page 21, lines 9-11). Consequently, the demonstration that extraembryonic tissue in developing embryos is a source of molecules capable of stimulating at least one of hematopoiesis, endothelial differentiation and endothelial cell proliferation is an important new finding. Applicants have provided descriptions of (a) tissues (page 38), (b) diffusible factors; (page 34) and (c) purified molecules derived from extra-embryonic tissue (page 44-47) to exemplify the claimed invention.

With respect to the Examiner's comments on page 6, line 1 of the office action concerning vascular growth, applicants have provided an example that demonstrates selective

enhanced expression of PECAM-1, flk-1 and Vezf-1. For example, applicants teach that these markers correspond to developing vasculature (Page 20, line 23-24; Page 39, line 9).

With respect to the Examiner's comments on page 6, lines 9-11 of the office action, applicants have amended the claims to more distinctly describe the invention. In light of these amendments, Applicants submit that this rejection is moot. The Examiner has further suggested that because the skilled artisan cannot envision the detailed chemical structure of the encompassed compounds, the above invention has not been reduced to practice. While this observation may be valid when a chemical composition is the subject of the claim, it is not applicable to method claims (see discussion below). The applicants' invention is directed to a method of stimulating a specific biological effect in specific target cells using a class of compounds derived from a specific tissue. With respect to the class of compounds, there is sufficient knowledge in the art to enable one of ordinary skill in the art to obtain any protein derived from a particular tissue. There is no analogy with the cases cited by the Examiner that are directed to composition of matters, more specifically to DNA molecules that are claimed by their functionality only.

Conception occurs when one " is able to define [a chemical] by its method of preparation" Amgen v. Chugai at 1021

The Examiner's assertion that detailed chemical structure is required for compounds used in a method claim is not supported by the cited cases. In Fiers v. Sugano, the claims in dispute in an interference were to DNA molecules. The Fiers disputed claim was to a DNA which consists essentially of a DNA which codes for a human fibroblast interferon beta polypeptide, while the Sugano claim was directed to a complete sequence for the beta - interferon gene. The Amgen Inc. v. Chugai decision was directed to composition of matter claims concerning DNA molecules. The Chugai claims were expressed in the form of a purification parameters, while the Amgen claim was directed to a DNA sequence encoding human erythropoietin. The Court in Amgen did not limit conception to envisioning chemical structure. In fact, Amgen at 1021, the court determined that conception occurs when one " is able to define [a chemical] by its method of preparation".

The Examiner has cited Fiddes v. Baird which concerned an interference between two parties and concerned a composition of matter claim for DNA encoding mammalian fibroblast growth factor (Fiddes). Baird was reported to have provided a description of a theoretical FGF sequence was incorrect as the basis for his composition claims and process method claims. According to expert witness testimony, Baird's theoretical bovine sequence differed from the naturally occurring bovine sequence by 106 out of 438 possible nucleotides. It is not surprising therefore that the judge concluded that Baird was not in possession of the bovine FGF let alone any other gene for mammalian FGF at the time of filing (p 1484). In contrast to Fiddes, applicants assert that the method of the claimed invention is practical and based on accurate information from a series of carefully designed experiments.

The applicants have adequately enabled the claimed invention by setting out in the specification the method and use of the claimed invention.

The Examiner has objected to the terminology "functionally equivalent to a gene product expressed in an embryo's extraembryonic tissue". (page 7, line 19 of the action). Applicants have amended the claims to more precisely define the invention and therefore this objection is now moot. However, applicants reserve the right to prosecute the full scope of the claims in a subsequent filing.

Applicants assert that the disclosure meets the enablement standard presented in In re Wands. In re Wands states that " Enablement is not precluded by the necessity for some experimentation such as routine screening." The specification provides a number of different assays which permit routine screening of compounds to determine whether they stimulate mesodermally derived cells to undergo at least one of hematopoiesis, endothelial differentiation or endothelial proliferation. In re Wands concerns a patent application that claimed monoclonal antibodies and was filed in 1980 shortly after the monoclonal antibody technology became available to the scientific community through the pioneering work of two British scientists. At that time, a hybridoma that produced monoclonal antibodies with a particular specificity was a rare event in a population of hybridomas. Not surprisingly, Wand found that only 4/143 hybridomas fell within the claims.

> The board concludes that Wands' low rate of success shows that a person skilled in the art would have to engage in undue experimentation in order to make antibodies that fall into the claims. (1405)

The circumstances of In re Wand are different from that of the applicants' invention. Applicants have shown that the extraembryonic tissue is necessary to cause development of blood islands at a certain stage in development and have demonstrated the activity of tissue, diffusible factors and compounds derived from extraembryonic tissue stimulate at least one of hematopoiesis, endothelial differentiation and proliferation. The Examiner has further pointed out that several compounds have been reported in the literature to influence vasculogenesis and hematopoiesis as demonstrated by knockout experiments in mice and associated loss of function. (Page 8, line 6 of the action). However, it is well recognized that the negative act of knocking out a gene and observing loss of function in a developing embryo is not sufficient to demonstrate the positive effect of a compound inducing a function. Furthermore, influence of a process determined by knock-out experiments as defined by loss of function does not fall within the scope of the claims because the claimed method is directed to a gain of function, namely a method of stimulating undifferentiated mesodermally derived cells to undergo at least one of hematopoiesis, endothelial differentiation and endothelial proliferation.

With respect to the Examiner's assertion that the predictability concerning whether a compound might induce hematopoiesis is low with respect to the cited prior art, applicants have provided data to demonstrate that contacting cells with an embryos's extraembryonic tissue derived compound will stimulate at least one of hematopoiesis, endothelial cell differentiation and endothelial cell proliferation. Example 2(c) describes a blastocyst assay for screening compounds that can stimulate hematopoiesis and endothelial cell proliferation and differentiation of undifferentiated mesodermal cells. Benzidine staining of blastosacs provided histological evidence for vascular channels and developing vasculature (page 42, lines 10-12 and figures 2, 3d). Example 3 describes a cell based assay for identifying the ability of compounds (and their functional equivalents) expressed in an embryo's extraembryonic tissue to stimulate undifferentiated mesodermal cells to undergo hematopoiesis along erythroid and myeloid lineages. Example 3 further shows how colony numbers increase for cells in the

hematopoietic pathway including cells of the myeloid lineage and cells of the erythroid lineage. (Page 45, lines 18-25 and Table 1). The role of compounds derived from the extraembryonic tissue for inducing hematapoiesis are shown in Figure 7 and 8 where induction of embryonic hematopoiesis occurs in the presence of extraembryonic tissue. Figure 9 demonstrates how a compound that is secreted by the extraembryonic tissue, exemplified by hedgehog, may stimulate hematopoiesis. Example 4 describes the inhibition of erythropoiesis by blocking antibodies of compounds derived from the extraembryonic tissue exemplified by hedgehog. Example 5 describes how receptors on undifferentiated mesodermal tissue that are responsive to compounds derived from extraembryonic tissue can be targeted by agonists or antagonists to regulate hematopoiesis. Example 6 describes how multiple compounds derived from an embryo's extraembryonic tissue may provide an enhanced stimulation of hematopoiesis exemplified by hedgehog protein and TGF-β proteins. The above examples provide extensive support for the claimed method. Although many (but not all examples) are directed to measuring hematopoiesis exclusively, applicants have pointed out the requirement for extraembryonic tissue in the formation of blood islands which require hematopoiesis , endothelial differentiation and proliferation and have further demonstrated activation of vascular endothelial cell markers in model systems. (Page 38, lines 23- page 39, line 30. Figure 16.3 which describe how vascular tissue can be induced by instructive signals from the visceral endoderm).

For example, the applicants have exemplified the claimed method in Figure 8-1, which shows the induction of hematopoiesis by visceral endoderm signals, Figure 16-2 which shows activation of hematopoietic markers in recombinant embryo explant cultures, and Figure 16-3 which shows activation of vascular endothelial cell markers (PECAM-1, flk-1 and Vezf-1) all of which are art recognized markers for vascular endothelial cells, in recombinant embryo explant cultures. The claimed method of the invention is further exemplified in Figure 15 which shows how extra embryonic ectoderm and visceral endoderm may be separated from the epiblast as a source of compounds to stimulate endothelial cell proliferation and differentiation or hematopoiesis.

The Examiner has rejected the claims as not enabled with respect to gene therapy. The protocols for gene therapy using a particular gene are well established. Nonetheless, the claims have been amended so that the rejection is now moot.

With respect to the Examiner's comments on page 9 line 20 to page 10, line19, the claims have been amended so as to render the rejection moot. The term "functionally equivalent to " is not present in the amended claim. Applicants thank the Examiner for pointing out the inconsistency of the definition of "synergistic effect". With respect to the Examiner's comment on the synergistic effect as defined in the specification, the definition has been removed from the specification so that the person ordinarily skilled in the art should interpret the term "synergistic effect" as a term of art. "Synergistic effect" as a term of art is derived from the Greek "synergos" which means "acting together". The term is used to describe the increased activity that arises from combining the activity of two or more agents when used together as compared to the cumulative effect of their separate activity. In Example 6 on page 49 of the application, synergistic activity is taught with the example of hedgehog and TGF-β. proteins.

Rejection under 35 U.S.C. § 112 second paragraph

Although applicants believe that the term "causing the compound to access the cells" is clear and definite in light of the specification, the claims have been amended to more distinctly describe the invention.

Summary

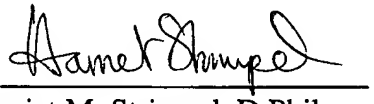
The claims as amended are supported in the specification, distinctly point out the invention and are enabled by the extensive supporting description. These claims include a method of stimulating a population of undifferentiated mammalian mesodermally derived cells to undergo at least one of hematopoiesis, endothelial cell differentiation and proliferation, that includes the steps of providing a first compound selected from the group consisting of hedgehog, WNT and optionally a second compound comprising a TGF-β (claim 57) ; a method of stimulating a population of undifferentiated mammalian mesodermally derived cells to undergo at least one of hematopoiesis, endothelial cell differentiation and proliferation that includes the

steps of contacting cells with an embryo's extraembryonic tissue derived compound and stimulating the cells to undergo at least one of hematopoiesis, endothelial differentiation and proliferation (claim 76). The claims as amended also includes a claim to a process for identifying a compound capable of the activity described above which includes: screening a library of compounds expressed by the extraembryonic tissue using assays developed by the client and described in the specification that include cultured mammalian epiblests, anterior ectoderm, blastocysts or embryoid bodies (claim 78).

Conclusion

For the above reasons, all claims presently in the application are believed to be allowable over the art of record and early notice to that effect is respectfully solicited. Applicants request the courtesy of a phone call from the Examiner to resolve any further outstanding issues if they arise so as to expedite the above case to allowance. Applicants petition for an extension of two months under 37 C.F.R.1.16 and request that the sum of $190 be charged to the Deposit No.19-4972. Please charge any additional fee required for the timely consideration of this application to the above identified deposit account.

Respectfully submitted,



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